Local adaptation and variation in life history reaction norms within the Icelandic cod stock

Lísa Anne Libungan

Faculty of Life and Environmental Sciences
University of Iceland
2009

t

LOCAL ADAPTATION AND VARIATION IN LIFE HISTORY REACTION NORMS WITHIN THE ICELANDIC COD STOCK

Lísa Anne Libungan

90 ECTS thesis submitted in partial fulfillment of a
Magister Scientiarum degree in Biology

Advisors
Prof. Guðrún Marteinsdóttir
Dr. Timothy B. Grabowski

Opponent
Theódór Kristjánsson, M.Sc., Ph.D Candidate
Project Manager at Stofnfiskur Hf

Faculty of Life and Environmental Sciences
School of Engineering and Natural Sciences
University of Iceland
Reykjavík, Desember 2009

Local adaptation and variation in life history reaction norms within the Icelandic cod stock
90 ECTS thesis submitted in partial fulfillment of a M.Sc. degree in Biology

Faculty of Life and Environmental Sciences
School of Engineering and Natural Sciences
University of Iceland
Askja, Sturlugötu 7
107 Reykjavík, Reykjavík
Iceland

Telephone: 525 4600

Bibliographic information:
Lísa Anne Libungan, 2009, Local adaptation and variation in life history reaction norms within the Icelandic cod stock, M.Sc. thesis, Faculty of Life and Environmental Sciences, University of Iceland.

Printing: Háskólaprent, Fálkagata 2, 107 Reykjavík
Reykjavík, Iceland, Desember 2009

Abstract

Increasing number of studies have shown that marine fish stocks, which are often composed of numerous spawning components, can adapt to local environmental conditions. The Icelandic cod stock is exposed to highly varied environmental conditions. In terms of temperature, the areas north of Iceland are influenced by cold Arctic Water while the southern areas are dominated by warm Atlantic Water. Cod is known to spawn all around the island and consequently larvae hatch into distinctly different environments, especially with respect to temperature. The main objective of this study was to explore the potential adaptation among larval cod exposed to different temperature. A common-garden experiment was conducted to evaluate the response in growth rates and survival among larvae originating from northern and southern located spawning grounds. Individuals from each location were reared for 60 days at three temperature treatments (4°C, 8°C, 12°C). Main results showed that northern and southern cod differed in their growth rate reaction norms and mortality as a response to different temperature treatments. At 12°C, the northern cod larvae grew significantly faster than the southern larvae. At 4°C this was reversed, where the southern cod larvae grew faster than the northern ones. However, it should be noted that the difference at 4°C was only marginally significant. No growth differences were detected at 8°C. In mortality, northern cod had significantly lower rates compared to southern when raised at 4°C. At 8°C populations did not differ in mortality, but in 12°C northern cod exhibited total mortality at the age of 35 days, while southern persisted throughout the experiment. Results indicate that northern and southern cod exhibit differences in physiology during their early life stages which suggests local adaptations to thermal conditions.

Útdráttur

Fjöldi rannsókna hafa sýnt að stofngerð fiska er oft flókin og samsett úr mörgum stofnum og undirstofnum sem einkennast af langtíma aðlögun einstaklinga að sínu nánasta umhverfi. Þorskurinn er lýsandi dæmi um slíkan fjölbreytileika en rannsóknir hafa sýnt að stofngerð hans einkennist af erfðafræðilega frábrugðnum undirstofnum og lífssöguhópum. Við Íslandsstrendur býr þorskurinn við breytileg umhverfisskilyrði. Á hafsvæðinu norður af landinu gætir áhrifa frá Arktískum kaldsjó en við suðurströndina er að finna hlýjan Atlantískan sjó. Hrygning á sér stað allt í kringum landið og klekjast þorsklirfurnar því við mismunandi umhverfisskilyrði, sérstaklega hvað varðar hitastig. Megintilgangur þessarar rannsóknar var að kanna aðlögun þorsklirfa að mismunandi hitastigi. Framkvæmd var tilraun þar sem borinn var saman vaxtarhraði og lifun þorsklirfa frá norður- og suðursvæði. Lirfurnar voru aldar upp aðskildar í 60 daga við þrjú hitastig (4°C, 8°C, 12°C). Niðurstöður sýndu að svörun þorsklirfa við mismunandi hitastigi hvað varðar vöxt og lifun, var ekki sá sami hjá þorsklirfum að sunnan og að norðan. Þannig uxu þorsklirfur að norðan hraðar en þær að sunnan við hátt hitastig (12°C) en hægar við lágt hitastig (4°C). Munurinn við 12°C var hámarktækur á meðan munurinn við 4°C var á jaðri þess að vera marktækur. Ekki fannst marktækur munur á milli norðurs og suðurs við 8°C. Dánartíðni þorsklirfa að norðan var lág við lægsta hitastigið en mjög há við hæsta hitastigið og lifði engin þeirra lengur en 35 daga við tólf gráður. Dánartíðni þorsklirfa að sunnan var hinsvegar há við lægsta hitastigið, og þær lifðu út tilraunina við hæsta hitastigið. Niðurstöður benda til þess að munur sé á vexti og lifun milli þorskungviðis frá norður- og suðursvæði við Ísland. Telja má líklegt að þessi munur sé tilkominn vegna svæðisbundinnar aðlögunar að breytilegu hitastigi.

Contents

Abbreviations

NC = Northern Cod
SC = Southern Cod
SE = Standard error
SD = Standard deviation
SGR = Specific growth rate
PSU = Practical salinity unit
μg = Micrograms
mmSL = Standard length in millimeters
HRS = Hours
HSI = Hepatosomatic index
ANOVA = Analysis of variance
MRI = The Marine Research Institude
MSE = The Marine Research Institude Experimental Station

Acknowledgements

I would like to start by thanking my supervisor Guðrún Marteinsdóttir for her knowledgeable guidance, for giving me this great opportunity and for being an inspiration to further my studies in the marine environment. My supervisor Timothy B. Grabowski, for helping me with sampling, for his fast response when revising this thesis, teaching me the ways in scientific writing and for keeping me on track, I give him my best gratitude.

Through my studies I was privileged to be a part of the research group MarICE. Thanks guys for all your support and fun times! Special thanks go to MarICE members: Bruce J. McAdam for helping me with sampling and statistics, Jónas P. Jónasson and Heidi E. Pardoe for help with sampling and useful discussions and Kai Logemann for sharing his wisdom on the hydrodynamics around Iceland. The Marine Research Institude is thanked for their contribution in making this research possible, and the great guys in the experimental station in Grindavík: Agnar Steinarsson, Matthías Oddgeirsson, Kristján Sigurðsson and Njáll Jónsson, thank you for maintaining the larvae, help and good advice. The captain and crew of Friðrik Sigurðsson and Þorleifur EA are thanked for their help.

My husband Kjartan Benediktsson gets my sincere gratitude for believing in me, helping me in every possible way and for being a constant source of motivation throughout my studies. Additional thanks go to: Snæbjörn Pálsson for sharing his statistical expertise on a number of occations, Theódór Kristjánsson for teaching me how to fertilize cod eggs and improving this thesis, Lilja Stefánsdóttir for helping me sample cod eggs in the north of Iceland and Rakel Guðmundsdóttir for many great talks in and outside Askja. The Icelandic Research Fund for Graduate Students are greatly thanked for their support.

1 Introduction

Historically, in the marine environment, local adaptation was believed to exist only on broad geographic scales because of a lack of strong barriers to gene-flow on smaller spatial scales. Increasing evidence suggests however that local adaptations do occur on fine geographic scales within marine fish populations. Marine fish populations can adapt to their local environment in various life history parameters, such as swimming performance (Hunt von Herbing 2002), egg size (Marteinsdóttir and Able 1992), growth rates and survival (Hutchings et al. 2007) and metabolic rates (Grabowski et al. 2009), allowing higher fitness under local conditions than populations originating from other locations. A common example of local adaptation in widely distributed species, is a latitudinal countergradient variation in growth (Conover and Present 1990). Generally, this growth variation is a response to a latitudinal gradient in water temperature such that individuals originating from high latitudes grow faster than conspecifics at lower latitudes, to compensate for a shorter growing season. By growing faster within the brief period of the year when high temperature occurs, high latitude forms reduce the risk of overwinter mortality (Conover and Present 1990). Evidence for countergradient variation has been reported to occur on relatively small geographic scales for various life history strategies of larval (Hunt von Herbing 2002, Hutchings et al. 2007), juvenile (Salvanes et al. 2004) and adult marine fish (Conover and Present 1990). Recent studies have found considerable variation in life history and genetic structuring in Atlantic cod populations at small geographic scales (Salvanes et al. 2004, Pampoulie et al. 2006, Knutsen et al. 2007, Arnason et al. 2009). Differences in survival (Hutchings et al. 2007), food conversion efficiency (Purchase and Brown 2001), condition (Pardoe et al. 2008) and metabolic rates (Grabowski et al. 2009) have also been reported on fine geographic scales between cod populations which occupy different environmental conditions. Variation between cod populations in growth has received some recent interest. Svasand et al. (1996) found growth performance to differ between Norwe-

gian coastal cod and Arcto Norwegian cod. Another study on Norwegian cod found evidence for countergradient variation in life history traits, including growth and feeding performance (Salvanes et al. 2004). Similarly, growth experiments of cod populations from the North American coast found that cod from a cold environment had better growth performance at colder temperatures and overall a broader range of temperature for optimal growth (Dutil et al. 2008). However, a recent study on juvenile cod from the same area did not find evidence for countergradient variation in growth, although growth patterns appeared genetically determined (Wijekoon et al. 2009).

Icelandic cod is a prime example of a marine fish showing diversification within a highly variable habitat despite a lack of obvious barriers to gene-flow. Substantial water temperature differences are found around the island, in particular between the northern and southern shelf regions which also differ interannually within each region (Fig 2.1, Marteinsdóttir et al. 2000, Malmberg and Valdimarsson 2003). Warm water is transported northwards by the Irminger Current along the southwestern, western and northern coasts, and by branches of the North Atlantic Current which move towards the southeastern coast of the country. From the north, the East Greenland Current brings cold and fresh Polar Water, while the East Icelandic Current brings cold Arctic Water (Malmberg and Valdimarsson 2003). The convergence of these warm and cold currents create strong thermal gradients northwest and east of the country, and their different contribution create highly variable hydrographic conditions around Iceland (Malmberg and Valdimarsson 2003, Logemann and Harms 2006). Variation has been found between cod occupying the northern and southern regions in microsatellites and gene frequency of the Pan I locus, with gene-flow discontinuities thought to be the result of different hydrographic conditions (Pampoulie et al. 2006). Tagging studies have confirmed spawning adult cod seldom migrate between the northeast and southwest regions (Pampoulie et al. 2006). Differences in size and age structure of adult cod have been reported between the northern and southern regions, where southern individuals are on average larger at age than their northern counterparts (Jónsdóttir et al. 2006, Jónsdóttir et al. 2008). The southern component initiates spawning in the middle of March and terminates it in the beginning of May, while the northern component spawns slightly later, from late April through May (Marteinsdóttir et al. 2000). Most cod larvae produced near and on the main spawning grounds in southern Iceland are carried with the Irminger Current to nursery grounds along the north coast (Marteinsdóttir

et al. 2000, Astthorsson et al. 1994). In the process, southern larvae experience a wide range of temperatures which are in general 2-4°C higher on the main spawning grounds on the southwest coast, compared to local temperatures within fjords on the west, north and east coasts (Marteinsdóttir et al. 2000). Larvae spawned in the north remain in more local locations than their southern counterparts, and typically experience a cooler and narrower temperature range of 3-6°C (Marteinsdóttir et al. 2000).

Due to the complex oceanographic conditions around Iceland, and variable contributions of water masses causing thermal variation, it is possible that adaptations might have arisen in growth and survival within the Icelandic cod stock during early life in response to these conditions. We conducted a common-garden experiment to evaluate differences in growth and survival between cod from the northern and southern regions over a 60 day period at three different temperatures (4°C, 8°C, 12°C) to test the hypothesis that any observed differences are due to adaptation to local thermal conditions. Local adaptation has been reported for other cod stocks in response to different thermal regimes. For example in Norwegian waters, coastal cod populations have been found to differ in growth rates (Salvanes et al. 2004) and variation in growth rates and survival has been reported among populations of Atlantic cod occupying Canadian waters (Hutchings et al. 2007) despite the lack of physical barriers for dispersal. The northern and southern Icelandic cod components have been found to be genetically distinct (Pampoulie et al. 2006), differ in otolith morphology (Jónsdóttir et al. 2006), condition (Pardoe et al. 2008), growth and maturity (Jónsdóttir et al. 2008, Marteinsdóttir and Begg 2002), time of spawning (Marteinsdóttir et al. 2000) and are locally adapted to their thermal regimes in metabolic rates (Grabowski et al. 2009). It is therefore reasonable to believe that local adaptation might have arisen in growth and survival during early life between the northern and southern cod components. As mentioned, cod in northern Iceland experiences colder waters compared to their southern counterparts (Marteinsdóttir et al. 2000, Astthorsson et al. 2007). If variation exists in growth and survival between northern and southern cod, it indicates they exhibit differences in physiology during their early life stages, suggesting local adaptations to thermal conditions. Confirming the presence of local adaptation in growth and survival between the northern and southern cod components would further confirm population structuring within the Icelandic cod stock, and suggest there exists a mechanism maintenance contributing to the population structuring within the stock.

2 Materials and methods

2.1 Sampling at sea

Spawning Icelandic cod were captured using gillnets deployed from commercial fishing vessels at spawning areas located northeast and southwest of Iceland (Fig 2.1). In the southwest, 12 female and 6 male running ripe southern cod (SC) were caught within the main spawning grounds at Selvogsbanki on 15 April (63°45´N, 20°57´W) and 16 April (63°32´N, 21°49´W) 2008. In the northeast, 24 female and 12 male

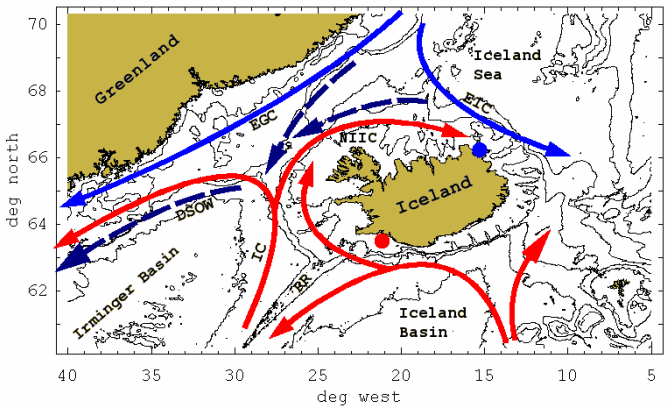

Figure 2.1: Bathymetry around Iceland and sampling areas of adult cod used in this study. The northern cod component was sampled from Þistilfjörður (blue circle) off northeast Iceland and the southern cod component from Selvogsbanki (red circle) off southwest Iceland. Red arrows: Atlantic Water (AW), blue arrows: Polar and Arctic Waters (PW) and dashed blue arrows: Denmark Strait Overflow Water (DSOW). Modified from Logemann and Harms (2006).

running ripe northern cod (NC) were caught at Þistilfjörður (66°10´N, 15°25´W) on 23 April 2008. Gametes were stripped and fertilized *in vitro* onboard the vessels.

Total length along with total, gutted and liver weights were recorded from each individual. Fertilized eggs from each male-female cross were kept in separate 1.0 L containers and stored on ice during the six hour transit to the rearing facilities at the Marine Experimental Station (MES) at Staður in Grindavík on the southwest coast of Iceland.

2.2 Common-garden experiment

Each male-female cross of fertilized eggs was incubated individually in a 25 L flow-through (0.5 L min^{-1}) hatching silos at the MES following a modified version of the cod rearing protocol described by Steinarsson (2004). In brief, silos were aerated and maintained at constant temperature (8.0 ± 1.0°C) and salinity (31.0 ± 0.05 PSU) during the two-week incubation period. Dead eggs were removed daily from each silo. Eggs were monitored closely to determine when 90-100% of the eggs had hatched and this was designated as day 0 post-hatch. On day 1 after hatching, NC larvae were pooled in one tank, and SC larvae in another. The density of fish was first measured in each holding tank by gently stirring the water and collecting ten 10 mL samples. This method was used to reduce the handling of the larvae, as previous experience had shown that more accurate counting transferring larvae individually between containers resulted in high mortality. Twelve batches of approximately 2000 larvae each were then drawn from each of the pooled tanks by measuring out the appropriate volume of water. These batches were each randomly assigned to one of three temperature treatment groups.

Treatment groups were moved to 150 L flow-through (0.5 L min^{-1}) rearing silos and maintained at one of three target temperatures: 4.0 ± 1.0°C, 8.0 ± 1.0°C and 12.0 ± 1.0°C, with four silos (replicates) at each temperature (Fig 2.2). Water temperature was monitored and recorded daily in all silos to the nearest 0.01°C. Actual mean temperatures in silos for NC resulted in: 4.11 ± 0.05°C, 8.02 ± 0.02°C, 12.24 ± 0.01°C, and for SC: 4.08 ± 0.04°C, 8.05 ± 0.02°C, 12.47 ± 0.02°C, and hereafter will be referred to as 4°C, 8°C and 12°C treatments. Larvae were acclimated to the coldest (4°C) and warmest (12°C) temperature treatments by gradually changing the temperature from 8°C over a period of four days, during which temperatures were held constant for the intermediate temperature group (8°C). Larvae were fed

to excess rotifers three times a day, in the morning, midday and evening, during the first 31 days. The first two feedings consisted of AlgaMac-3000 (Aquafauna Bio-Marine Inc., Hawthorne, USA) enriched rotifers and the evening feed included AlgaMac enhance enriched rotifers. Following Marcil et al. (2006) and Hutchings et al. (2007), larvae were fed a 1:1 mixture of *Artemia* nauplii and rotifers from day 32 to 39 post-hatch and only *Artemia* nauplii from day 40 to 60.

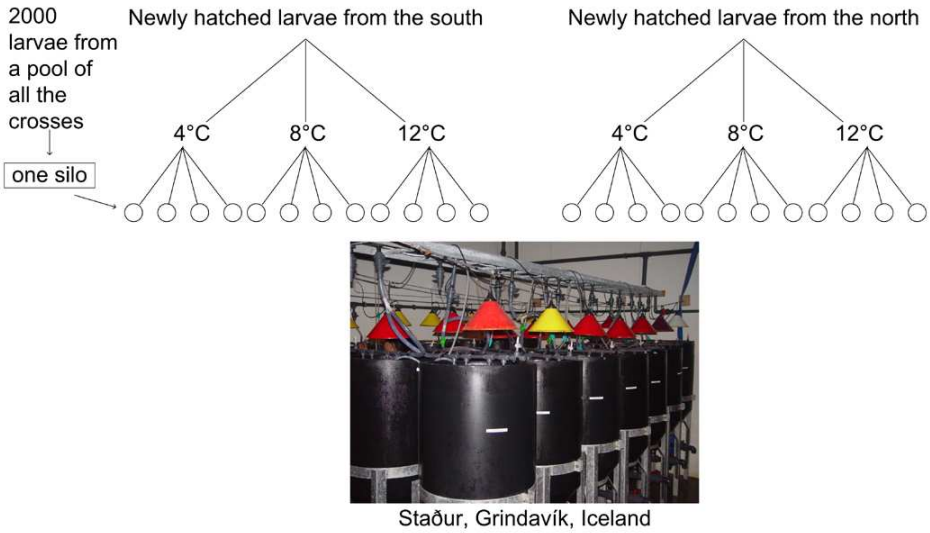

Figure 2.2: The experimental design of the common-garden experiment used in this study. For each cod component 2000 newly hatched larvae were placed in three different temperatures (4°C, 8°C and 12°C), with four replicates/silos (circles) for each temperature treatment, and raised over a 60 day period in the Marine Research Institude experimental station at Staður, Grindavík, Iceland.

2.3 Sampling eggs and larvae

Eggs from each population were sampled on the third day after fertilization and dry weight and diameter were recorded following the procedure described by Marteinsdóttir and Steinarsson (1998). After hatching, 30 larvae were sampled at random from each rearing silo on days 1, 12, 21, 30, 46 and 60 post-hatch for each population to obtain length and weight measurements. Larvae were euthanized with tricaine methanesulphonate (MS-222) and rinsed with freshwater to remove salt. A Leica MZ95 stereomicroscope (Leica Micro-systems, Wetzlar, Germany) with a Evolution LC-PL A662 camera (MediaCybernetics, Maryland, USA) was used to capture digital images of larvae from each silo using Optimas 6.5 (MediaCybernetics, Maryland, USA) to obtain standard length measurements. Length measurements were calibrated using a digital image of a micrometer for each magnification. Larvae were placed on a numbered and pre-weighed piece of plastic plankton net (five larvae on each piece), dried in a drying oven at 48°C for 24 hrs and placed in a desiccator for an additional 24 hrs. Finally, each plastic net was weighed again to obtain the average dry weight of five fish to the nearest 0.001 μg with a Orion Cahn C-34 microbalance (THERMO, Beverly, Massachusetts, USA), calibrated before each sampling event. Larvae were counted by hand in all silos on days 35 and 60 post-hatch to estimate mortality.

2.4 Data analysis

Potential pre-existing differences between NC and SC, due to maternal effects and intrinsic differences in egg size and weight, and larval size and weight at hatching, were evaluated using two-sample t-tests. One-way ANOVA was used to test for differences in mean temperatures in silos to meet common-garden assumptions of homogeneity of treatments between populations, and silos were excluded where necessary to meet those assumptions. Differences in mean length, gutted weight, adjusted hepatosomatic index (HSI):

$$\text{HSI} = \frac{\text{Liver weight}}{\text{Gutted weight}} \times 100$$

and adjusted Fulton's K condition factor:

$$K = \frac{\text{Gutted weight}}{\text{Length}^3} \times 100$$

of adult fish were compared between NC and SC. When condition indices were calculated, gutted weight was used rather than total weight to ensure a more accurate assessment of condition since feeding intensity and gonad maturation can vary significantly between individuals, locations and years (Lambert and Dutil 1997).

Differences in growth rates between NC and SC were assessed using specific growth rates (SGR, % per day). SGR were calculated for each silo as change in average larval length over days following a modified procedure described by Dutil et al. (2008) and using the equation:

$$\text{SGR} = \frac{ln(L_{i+1}) - ln(\bar{L}_i)}{(t_{i+1} - t_i)} \times 100$$

where L_i is standard length (mm, estimated from digital images) at time t_i (days) and $\bar{L}_i$ is the mean length of larvae at time t_i. The same equation was used to calculate SGR in dry weight.

A linear mixed effects model with silos (replicates) incorporated as a random effect was used to test for population $\times$ temperature interactions for both length- and weight-based SGR reaction norms when larvae were at ages 12-30 days after hatching and to test the null hypothesis that NC and SC exhibited the same SGR (Kutner et al. 2005). The age 12 days after hatching was chosen as the starting point for SGR calculations to be sure that larvae had absorbed their yolk sack, and the age 30 days after hatching as the end point, to estimate growth rates before larvae entered metamorphosis.

Changes in larval length and weight with temperature and age were analysed for possible differences in SGR between populations over the entire experimental period and digital images were examined to see when larvae were in metamorphosis. Metamorphosis of larval cod can be evaluated visually when outer characteristics of the larvae change, where the larval finfold transforms into functioning fins and swimming characteristics of the fish has been attained (Pedersen and Falk-Petersen 1992). This transformation begins when cod larvae are at 11-15 mmSL and is com-

pleted at approximately 20 mmSL (Pedersen and Falk-Petersen 1992).

Mortality rates were obtained by calculating the instantaneous mortality rate (% per day) over two time periods, 1-35 days and 35-60 days, assuming it was constant over the time period and accounting for the additional mortality from sampling. Two-way ANOVAs were conducted for each of the three temperature treatments separately to compare mortality rates between populations. When differences were found in mortality rates between populations, Tukey's multiple comparison was used to compare the two populations at each age period and temperature treatment. Statistical analyses were conducted with R (v. 2.6.2 R Development Core Team 2008). An α level of 0.05 was used to test for significant differences. Means and parameter estimates are presented $\pm$ 1 standard error (se). Length and weight data were log-transformed to meet parametric assumptions of normality. Where t-tests were used, data were tested for equality of variances with F-tests by testing the ratio of the group variances (Dalgaard 2002). Two-sample t-tests were used when variances were unequal and Welch two-sample t-tests when variances were equal (Dalgaard 2002).

3 Results

3.1 Female, egg and larval sizes

Pre-existing differences between NC and SC were observed among female spawners and early developmental stages of eggs and larvae (Table 3.1). Adult female SC were longer (t=-4.48, df=28.13, P=0.0001), heavier (t=-5.73, df=30.10, P<0.0001), and in better condition as measured by both the hepatosomatic index (HSI) (t=-5.15, df=16.08, P<0.0001) and Fulton's K (t=-3.06, df=17.47, P=0.007) than NC females. However, after fertilization, NC eggs had a greater diameter (t=9.75, df=241.49, P<0.0001) and were heavier (t=78.08, df=33.41, P<0.0001) than those from SC. While there was no difference in weight (t=0.50, df=25, P=0.62), NC larvae were longer than SC larvae (t=8.55, df=195, P<0.0001) at hatching (age 1-day).

Table 3.1: Mean size and condition of adult females, eggs and larvae from the two cod populations ± 1 sd. Stars (*) refer to significant differences from t-tests between the two populations for each of the variables ($P < 0.01$, **$P < 0.001$, ***$P < 0.0001$).*

	Northern cod	**Southern cod**
Maternal length (cm)***	82 ± 16	98 ± 5
Maternal weight (g)***	4200 ± 500	8115 ± 1572
Hepatosomatic index***	4.6 ± 1.7	8.1 ± 1.8
Fulton's *K* condition factor*	0.70 ± 0.07	0.85 ± 0.10
Egg diameter (mm)***	1.46 ± 0.03	1.37 ± 0.02
Egg dryweight (mg)***	0.136 ± 0.017	0.106 ± 0.006
Larval length at hatch (mm)***	4.93 ± 0.24	4.63 ± 0.25
Larval dryweight at hatch (mg)***	0.061 ± 0.032	0.055 ± 0.017

3.2 Variation in temperature among experiment units

Mean temperature did not differ significantly between silos at either 4°C (F=0.27, df=1, 413, P=0.60) or 8°C (F=1.89, df=1, 379, P=0.17) when the common-garden assumption for equality in the temperature experienced by each population was tested. However, temperatures differed among the 12°C silos (F=21.99, df=1, 163, P<0.0001), where one silo containing the northern population was too cold and one silo containing the southern population was too warm. To meet common-garden assumptions, these two 12°C silos (one from each population) had to be excluded from the experiment (after exclusion: F=0.33, df = 1, 122, P=0.57).

3.3 Specific growth rate reaction norms of cod larvae

Evidence of differences in larval growth between populations where revealed by examining the shapes of reaction norms in response to all temperature treatments when larvae were between ages 12 to 30 days after hatching (Fig 3.1). Populations responded differently to temperature as indicated by a significant population $\times$ temperature interaction in length-based SGR reaction norms (Fig 3.1a, P=0.03). Northern larvae had higher growth rates than southern at the highest temperature treatment (P=0.03), while populations did not differ in 4°C and 8°C (P>0.12). In weight-based SGR, populations showed a marginal difference at 4°C where southern larvae grew faster than northern (Fig 3.1b, P=0.07), while populations did not differ at 8°C or 12°C (P>0.25).

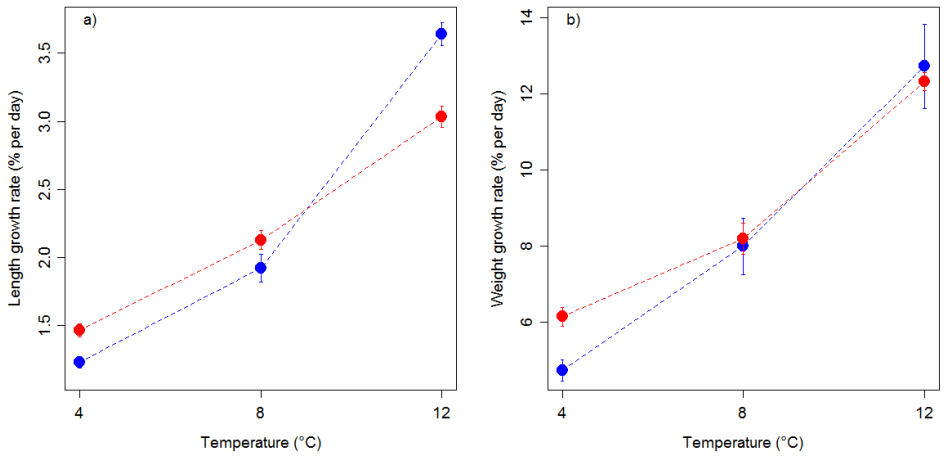

Figure 3.1: Specific growth rate reaction norms in a) length and b) weight for ages 12 to 30 days for larval cod from two components (blue: northern, red: southern) originating from different temperature regimes in Icelandic waters. Figures show population $\times$ temperature (4° C, 8° C and 12° C) interactions in SGR reaction norms.

3.4 Specific growth rate over time and metamorphosis of larval cod

Progressive changes in specific growth rates are likely to be linked with the ontogenetic changes that the larvae are experiencing; including metamorphosis. The metamorphosis transformation varied among the two populations depending on temperature treatment (Fig 3.2). In the 4°C treatment, neither population underwent metamorphosis (Fig 3.2a,d). In 8°C, both populations had initiated metamorphosis after 46 days, and had completed it by the age of 60 days (Fig 3.2b,e). At 12°C, only SC underwent metamorphosis and this started at the age of 30 days and was completed by the age of 46 days (Fig 3.2c,f).

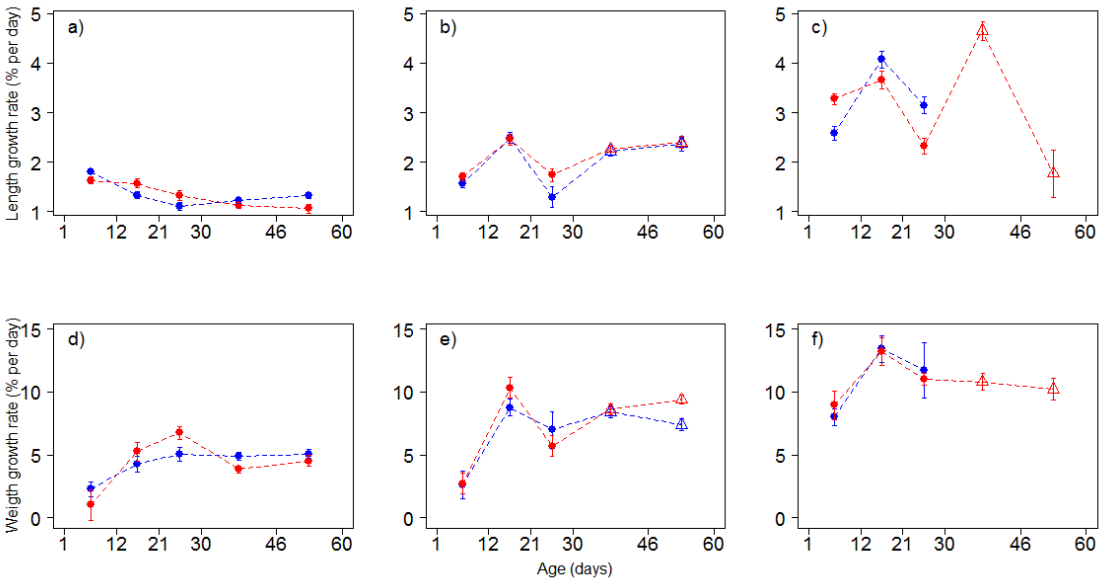

Figure 3.2: Specific growth rates of larval cod from two populations originating from different temperature regimes in Icelandic waters. Specific growth rates are shown for a)-c) length and d)-f) weight with ± 1 se as a function of age (days) and temperature treatment 4° C (a,d), 8° C (b,e) and 12° C (c,f). Blue: northern larvae, red: southern larvae. Circles around means represent larval cod that have not metamorphosed, triangles represent larvae that have metamorphosed. None of the NC larvae survived passed 35 days in the 12° C treatments, explaining the gap in the dataset in figures c) and f) for ages 35 to 60 days.

Northern cod initiated metamorphosis at the same time as SC in 12°C, but seemed to be unable to complete it and experienced complete mortality by the age of 35 days (Fig 3.2c,f). At 4°C, both NC and SC exhibited decreased length-based SGR during the first 30 days and in fact for SC this continued throughout the experiment (Fig 3.2a), while at the same time both populations simultaneously increased their weight-based SGR (Fig 3.2d). From 30-60 days, NC increased in length-based SGR, but their weight-based SGR stayed at constant. SC exhibited a decline in weight-based SGR from 30-46 days, but an increase from 46-60 days (Fig 3.2d). At 8°C, populations increased in both length- and weight-based SGR during the first 21 days (Fig 3.2b,e), but then NC exhibited a more extreme drop in length-based SGR than SC between ages 21-30 days (Fig 3.2b), with only minor differences thereafter. In the same temperature treatment, populations showed similar trends in weight-based SGR (Fig 3.2e) as in length-based SGR (Fig 3.2b), with a decline in weight while undergoing metamorphosis and an increase from 30-46 days, but seemed to diverge at age 46-60 days, with SC larvae increasing in weight-based SGR, but NC decreasing (Fig 3.2e). In the 12°C treatment, populations showed an increase in both length- and weight-based SGR during the first 21 days (Fig 3.2c,f) and a decline from ages 21-30 days when entering metamorphosis. As mentioned, is seems that NC larvae had started to metamorphose at the age of 30 days, but were unable to complete it due to total mortality at the age of 35 days, while SC larvae had initiated metamorphosis at 30 days and completed it by day 46. In 12°C, SC also exhibited increased SGR from 30-46 days, but a decline from 46-60 days (Fig 3.2c). According to the weight-based SGR, SC exhibited a decline from the start of metamorphosis at day 30 to the end of the experiment at day 60 (Fig 3.2f).

3.5 Mortality rates of larval cod

Populations showed differences in mortality rates which depended on age and temperature treatment (Fig 3.3). At ages 1 to 35 days, mortality rates of SC in the 4°C treatment were greater than those of NC (Fig 3.3a, F=3.58, df =1, 9, P=0.004). In all other age/temperature combinations, no differences were detected in mortality rates between NC and SC (Fig 3.3b,c, F<4.22, P>0.21). In the 12°C treatment at ages 35 to 60 days (Fig 3.3c), NC larvae did not survive beyond 35 days, while SC persisted throughout the experiment and exhibited low mortality rates (<0.0002% per day), but had a low number of surviving individuals.

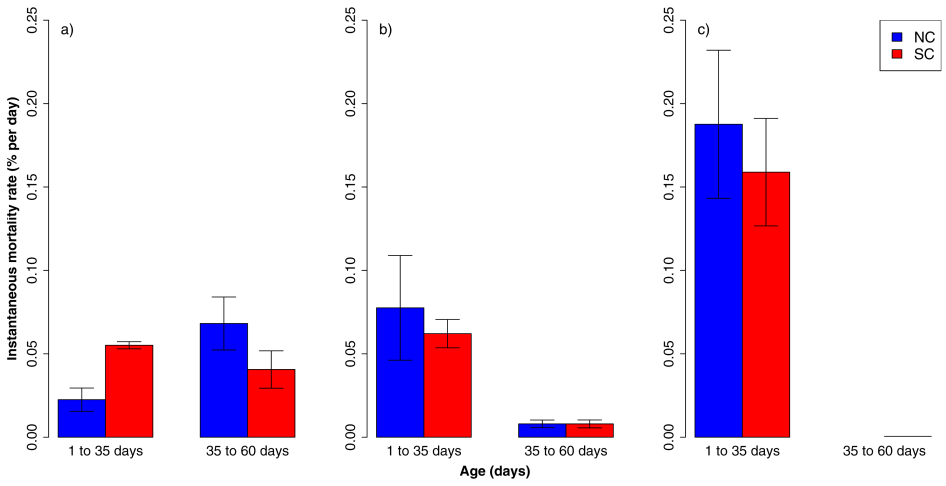

Figure 3.3: Mortality rates for larval cod from two cod populations (NC and SC) from different temperature regimes north and south of Iceland. Mortality rates with ± 1 se are shown for three temperature treatments: a) 4° C, b) 8° C and c) 12° C for two age periods: 1 to 35 days and 35 to 60 days. Southern larvae exhibited low mortality rates (<0.0002% per day) but had a low number of surviving individuals, shown with a single line in (c) at ages 35 to 60 days in the 12° C treatment, whereas NC larvae did not survive past 35 days.

4 Discussion

Cod larvae from northern (NC) and southern (SC) Icelandic waters raised in a common-garden environment differed in growth and mortality as a response to different temperature treatments. This would suggest there is an underlying genetic basis to the stock structure of Icelandic cod manifested by differences in life history traits in response to differing thermal regimes in the northern and southern regions of Iceland.

Larval growth varied between NC and SC depending on temperature treatment. By examining the shapes of reaction norms in response to temperature treatment, NC was observed to have significantly higher length-based specific growth rates (SGR) than SC at the highest temperature (12°C). In weight-based SGR, SC had higher growth rates than NC in the coldest temperature (4°C), but this relationship was marginally significant. These results do however show a pattern which is consistent with fish populations that exhibit countergradient variation in growth in response to a latitudinal gradient in temperature. At higher latitudes, the variation in growth rate is an evolutionary response by fish to grow faster to compensate for a shorter growing season compared to conspecifics from low latitudes at higher temperatures (Houde 1989, Conover and Present 1990, Conover and Schultz 1995). Evidence of adaptation to temperature and length of the growing season in terms of growth have previously been documented from common-garden experiments for various fish stocks, such as Atlantic halibut (Jonassen et al. 2000), Atlantic silversides (Conover and Present 1990) and Atlantic cod (Purchase and Brown 2001), where the fish at high latitude grew faster than those at low latitudes during the brief period of the year when high temperature occured, and also because the capacity for growth increases with latitude (Conover and Present 1990). As mentioned, due to variable water temperatures around Iceland, larvae spawned in the south of Iceland are on average exposed to higher temperatures compared to local temperatures within fjords on the west, north and east coasts during the spawning season

(Marteinsdóttir et al. 2000). Local retention of eggs and larvae within a fjord system (Knutsen et al. 2007) could be contributing to the growth variation observed between SC and NC larvae in this study. Atlantic cod within fjords in Norway has small geographically scaled population structuring, thought to be driven by mechanisms such as homing of adult to their natal spawning grounds and local retention of pelagic eggs and larvae (Knutsen et al. 2007). Knutsen et al. (2007) found a pattern consistent with former detected genetic structuring of adult cod (Knutsen et al. 2003) where cod eggs were found at higher densities within sheltered fjord basins compared to locations offshore. With Icelandic cod showing high spawning site fidelity (Thorsteinsson and Marteinsdóttir 1992, 1993), and the north is characterised by fjords while the south is not, retention of cod eggs and larvae spawned within fjords might cause adaptation to such conditions to arise. The growth differences observed in this study are therefore likely to represent intrinsic differences between the populations in terms of how they respond to temperature, and temperature may be acting as an isolating mechanism between the northern and southern stock components, allowing adaptations to local conditions to be maintained.

The metamorphosis transformation differed between NC and SC depending on temperature treatment. Neither NC nor SC metamorphosed at the coldest temperature of 4°C, but metamorphosed at the same time in the 8°C treatment. At 12°C, there was variation to the extent that SC larvae were able to metamorphose while NC exhibited total mortality before entering metamorphosis. Length of larval cod are one of the factors which control when they enter metamorphosis and starts when they are approximately 11-15 mmSL in length and completed when they reach 20 mmSL (Pedersen and Falk-Petersen 1992). By examining digital images and length measurements, it was apparent that neither NC nor SC had started to metamorphose at the age of 30 days and average length of 12.4 mmSL for NC, and 11.6 mmSL for SC. Only SC was able to complete the transformation at the age of 46 days and 24.7 mmSL, while NC exhibited total mortality at the age of 35 days. It is likely that NC had not yet attained the appropriate size to start the transformation when they exhibited total mortality.

Mortality varied between NC and SC at 4°C and 12°C, but not at 8°C. Northern cod larvae had significantly higher survival at the coldest temperature, while SC had higher survival at the highest temperature. These mortality differences likely represent an evolutionary trade-off between growth and survival under different temperature regimes. Rapid growth during a short growing season usually reduces the

risk of overwinter mortality (Conover and Present 1990). However, it is unlikely that NC would encounter water temperature of 12°C in its first weeks of life in the north, since water temperatures there do not approach 6-7°C until the end of May or beginning of June (Marteinsdóttir et al. 2000). Southern cod might have better tolerance for long exposure to higher temperatures, because during the larval stage in nature, they are exposed to higher temperatures than their northern counterparts (Marteinsdóttir et al. 2000). However due to low sample size of SC after the age of 35 days in the 12°C treatment, this warrants further investigation.

Although the common-garden experimental design was able to maintain constant environmental conditions, one aspect we did not control for were parental differences e.g. maternal effects. Generally, larger maternal size and better condition translates to larger eggs and larvae at hatch (Kjesbu 1989, Trippel et al. 1997, Marteinsdóttir and Steinarsson 1998). Northern cod adults used in this study were smaller than SC, and had significantly lower condition as measured by Fulton's K and lower hepatosomatic index (HSI), but had larger and heavier eggs, and larger larval size at hatch. This inconsistency between former studies and the present one is most likely an artifact of the time at which each population was sampled. Female cod produce several batches of eggs over a prolonged spawning season and generally experience a decrease in egg size through the season (Kjesbu et al. 1992, Chambers and Waiwood 1996, Marteinsdóttir and Steinarsson 1998). It is likely that SC adults, which were sampled on 15-16 April, and spawn on average from mid-March through April (Marteinsdóttir et al. 2000), was sampled further along in their spawning season than NC. Northern cod were sampled on 23 April, but are known to spawn on average from late April through May (Marteinsdóttir et al. 2000). This could explain the larger eggs and larval size at hatch for NC. Although size and condition of spawning females has been found to affect the size at hatch and viability of larval fish (Marteinsdóttir and Steinarsson 1998, Marteinsdóttir and Begg 2002), these effects are likely to only minimally influence the population effects on growth rates and larval survival in a common-garden environment, which have previously been reported being a consequence of genetic rather than maternal effects (Hutchings et al. 2007).

The findings of this study support previous conclusions regarding population structure within the Icelandic cod stock. As mentioned, genetic differences do exist between northern and southern Icelandic cod (Pampoulie et al. 2006). Life history strategies employed by Icelandic cod also differs in various ways between cod in the

northern and southern regions. Difference in behavior in terms of different migration patterns has been documented for Icelandic cod, where individuals which were tagged in the southwest region seldom migrated to the northeast region and vice versa (Palsson and Thorsteinsson 2003, Pampoulie et al. 2006). Otolith shape discriminates between adult cod originating from the north and south of Iceland, and among cod spawning below and above 125 m at spawning locations south of Iceland (Jónsdóttir et al. 2006). Icelandic cod from the northeast and southwest have been found to differ in physiology in terms of weight-specific oxygen consumption rates (VO_2/M) in response to abrupt temperature changes, where cod juveniles from the southwest region exhibited a greater decrease in VO_2/M when moved from warmer temperatures to colder, and northeast individuals had higher VO_2/M when moved from colder temperatures to higher (Grabowski et al. 2009). Life history diversity has been found in growth rates in adult Icelandic cod from different feeding regimes (Pardoe et al. 2009) and condition as measured in HSI from different temperature regimes, where condition resulted higher for cod from the north than south (Pardoe et al. 2008).

The present study was carried out in a common-garden environment where cod from the northern and southern regions where held under identical conditions, which suggests that the differences observed, in terms of growth and survival of cod larvae, have an underlying genetic basis reflecting adaptation to local conditions. These findings support previous conclusions that Icelandic cod employs a complex population structure (Marteinsdóttir et al. 2000, Jónsdóttir et al. 2006, Pampoulie et al. 2006, 2008, Arnason et al. 2009, Grabowski et al. 2009) and indicates that temperature might be more pronounced in maintaining life history diversity within the stock than previously anticipated. Further studies addressing the functionality of environmental factors on life history diversity would give valuable insight into how pronounced they are in maintaining stock structure within species, and give important implications for determining future management directions on managed stocks.

Bibliography

[1] Arnason, E., Hernandez, U.B., and Kristinsson, K. 2009. Intense Habitat-Specific Fisheries-Induced selection at the molecular *Pan* I locus predicts imminent collapse of a major cod fishery. PLoS ONE 4 (5): e5529, doi:10.1371/journal.pone.0005529.

[2] Astthorsson, O.S., Gislason, A. and Gudmundsdottir, A. 1994. Distribution, abundance, and length of pelagic juvenile cod in Icelandic waters in relation to environmental conditions. ICES J Mar Sci Symp. 198: 529-541.

[3] Astthorsson, O.S., Gislason, A., and Jonsson, S. 2007. Climatic variability and the Icelandic marine ecosystem. Deep-Sea Research II. 54: 2456-2477.

[4] Chambers, R.C. and Waiwood, K.G. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can J Fish Aquat Sci. 53: 1986-2003.

[5] Conover, D.O. and Present, M.C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia. 83: 316-324.

[6] Conover, D. and Schultz, E. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecol. and Evol. 10: 248-252.

[7] Dalgaard, P. 2002. Statistics and Computing - Introductory Statistics with R. Springer, New York, USA. 267 p.

[8] Dutil, J.D., Jabouin, C., Larocque, R. Desrosiers, G. and Blier, P.U. 2008. Atlantic cod (*Gadus morhua*) from cold and warm environments differ in their maximum growth capacity at low temperatures. Can J Fish Aquat Sci. 65: 2579-2591.

[9] Grabowski, T.B., Young, S.P., Libungan, L.A., Steinarsson, A. and Marteinsdóttir, G. 2009. Evidence of phenotypic plasticity and local adaption in metabolic rates between components of the Icelandic cod (*Gadus morhua* L.) stock. Environ Biol Fish. 86: 361-370.

[10] Houde, E.D. 1989. Comparative growth, mortality and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin, U.S. 87: 471-495.

[11] Hunt von Herbing, I. 2002. Effects of temperature on larval fish swimming performance: the importance of physics to physiology. J Fish Biol. 61: 865-876.

[12] Hutchings, J.A., Swain, D.P., Rowe, S., Eddington, J.D., Puvanendran, V. and Brown, J.A. 2007. Genetic variation in life-history reaction norms in marine fish. Proc R Soc Lond B Biol Sci. 274: 1693-1699.

[13] Jónsdóttir, I.G., Marteinsdóttir, G. and Pampoulie, C. 2008. Relation of growth and condition with the *Pan* I locus in Atlantic cod (*Gadus morhua* L.) around Iceland. Mar Biol. 154: 867-874.

[14] Jónsdóttir, I.G., Campana, S.E. and Marteinsdóttir, G. 2006. Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua* L.). ICES J Mar Sci. 63: 1501-1512.

[15] Jonassen, T.M., Imsland, A.K., Fitzgerald, R., Bonga, S.W., Ham, E.V., Naevdal, G., Stefansson, M.O. and Stefansson, S.O. 2000. Geographic variation in growth and food conversion efficiency of juvenile Atlantic halibut related to latitude. J Fish Biol. 56: 279-294.

[16] Kjesbu, O.S. 1989. The spawning activity of cod *Gadus morhua* L. J Fish Biol. 34: 195-206.

[17] Kjesbu, O.S., Kryvi, H., Sundby, S. and Solemdal, P. 1992. Buoyancy variations in eggs of Atlantic cod (*Gadus morhua* L.) in relation to chorion thickness and egg size: theory and observations. J Fish Biol. 41: 581-599.

[18] Knutsen, H., Jorde P.E., André, C., and Stenseth, N.C. 2003. Finescaled geographic population structuring in a highly mobile marine species: the Atlantic cod. Mol Ecol. 12: 385-394.

[19] Knutsen, H., Olsen, E.M., Ciannelli, L., Espeland, S., Knutsen, J.A., Simonsen, J.H., Skreslet, S. and Stenseth, N.C. 2007. Egg distribution, bottom topography and small-scale cod population structure in a coastal marine system. Mar Ecol Prog Ser. 333: 249-255.

[20] Kutner, M., Nachtsheim, C., Neter, J. and Li, W. 2005. Applied Linear Statistical Models, 5th ed. McGRAW-HILL, Singapore, Asia. 1396 p.

[21] Logemann, K. and Harms, I. 2006. High resolution modelling of the North Icelandic Irminger Current (NIIC). Ocean Sci. 2: 291-304.

[22] Malmberg, S.A. and Valdimarsson, H. 2003. Hydrographic conditions in Icelandic waters, 1990-1999. ICES J Mar Sci Symp. 219: 50-60.

[23] Marcil, J., Swain, D.P. and Hutchings, J.A. 2006. Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.). Biol J Linn Soc. 88: 351-365.

[24] Marteinsdóttir, G. and Able, K.W. 1992. Influence of egg size on embryos and larvae of *Fundulus heteroclitus* (L.). J Fish Biol. 41: 883-896.

[25] Marteinsdóttir, G. and Begg, G.A. 2002. Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser. 235: 235-256.

[26] Marteinsdóttir, G., Gunnarsson, B. and Suthers, I.M. 2000. Spatial variation in hatch date distributions and origin of pelagic juvenile cod in Icelandic waters. ICES J Mar Sci. 57: 1184-1197.

[27] Marteinsdóttir, G. and Steinarsson, A. 1998. Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. J Fish Biol. 52: 1241-1258.

[28] Pálsson, Ó.K., and Thorsteinsson, V. 2003. Migration patterns, ambient temperature, and growth of Icelandic cod (*Gadus morhua*): evidence from storage tag data. Can J Fish Aquat Sci. 60: 1409-1423.

[29] Pampoulie, C., Jakobsdóttir, K.B., Marteinsdóttir, G. and Thorsteinsson, V. 2008. Are vertical behaviour patterns related to the Pantophysin locus in the Icelandic cod (*Gadus morhua* L.)? Behav Genet. 38: 76-81.

[30] Pampoulie, C., Ruzzante, D.E., Chosson, V., Jörundsdóttir, T.D., Taylor, L., Thorsteinsson, V., Daníelsdóttir, A.K. and Marteinsdóttir, G. 2006. The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the *Pan* I locus, and tagging experiments. Can J Fish Aquat Sci. 63: 2660-2674.

[31] Pardoe, H., Thordarson, G. and Marteinsdóttir, G. 2008. Spatial and temporal trends in condition of Atlantic cod *Gadus morhua* on the Icelandic shelf. Mar Ecol Prog Ser. 362: 261-277.

[32] Pardoe, H., Vainikka, A., Thordarson, G., Marteinsdóttir, G. and Heino, M. 2009. Temporal trends in probabilistic maturation reaction norms and growth of Atlantic cod (*Gadus morhua*) on the Icelandic shelf. Can J Fish Aquat Sci. 66: 1719-1733.

[33] Pedersen, T. and Falk-Petersen, I.B. 1992. Morphological changes during metamorphosis in cod (*Gadus morhua* L.), with particular reference to the development of the stomach and pyloric caeca. J Fish Biol. 41: 449-461.

[34] Purchase, C., and Brown, J. 2001. Stock-specific changes in growth rates, food conversion efficiencies, and energy allocation in response to temperature change in juvenile Atlantic cod. J Fish Biol. 58: 36-52.

[35] R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

[36] Salvanes, A.G.V., Skjæraasen, J.E., and Nilsen, T. 2004. Sub-populations of coastal cod with different behaviour and life-history strategies. Mar Ecol Prog Ser. 267: 241-251.

[37] Svåsand, T., Jørstad, K.E., Otterå, H. and Kjesbu, O.S. 1996. Differences in growth performance between Arcto-Norwegian and Norwegian coastal cod reared under identical conditions. J Fish Biol. 49, 108-119.

[38] Steinarsson, A. 2004. Hatchery production of cod. In: Cod farming in Iceland (eds. Björnsson, B. and Gunnarsson, V.I). The Marine Research Institute in Iceland. Report 111: 41-86. [In Icelandic]

[39] Thorsteinsson, V. and Marteinsdóttir, G. 1992. Þorskmerkingar við Norðaustur- og Austurland vorið 1991 og endurheimtur sama ár. [Mark-recapture experiments at the northeast and east coasts of Iceland 1991.] Ægir. 85: 60-64. [In Icelandic]

[40] Thorsteinsson, V. and Marteinsdóttir, G. 1993. Þorskmerkingar í Stöðvarfirði og Gunnólfsvík 1991 og 1992 og endurheimtur úr þeim til ársloka 1992. Ægir, 2: 3-10. [In Icelandic]

[41] Trippel, E.A., Kjesbu, O.S. and Solemdal, P. 1997. Effects of adult age and size structure on reproductive output in marine fishes. In: Early Life History and Recruitment in Fish Populations (Chambers, R. C. and Tripple, E. A. eds). London: Chapman & Hall. 632 p.

[42] Wijekoon, M.P., Puvanendran, V., Ings, D.W. and Brown, J.A. 2009. Possible countergradient variation in growth of juvenile cod *Gadus morhua* from the northwest Atlantic. Mar Ecol Prog Ser. 375: 229-238.